Campylobacter pinnipediorum sp. nov., isolated from pinnipeds, comprising Campylobacter pinnipediorum subsp. pinnipediorum subsp. nov. and Campylobacter pinnipediorum subsp. caledonicus subsp. nov.

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Running title

Campylobacter pinnipediorum sp. nov.

Contents category

New taxa (Proteobacteria)

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The GenBank accession numbers for the whole genome sequences of strains RM17260, RM17261, RM17262, M203/00/3, M302/10/6 and M341/11/05 are CP012546, CP012547, CP012548, MBGA00000000, CP017018 and CP017258, respectively.

Summary

During independent diagnostic screenings of otariid seals in California (US) and phocid seals in Scotland (UK), *Campylobacter*-like isolates, which differed from the established *Campylobacter* taxa, were cultured from abscesses and internal organs of different seal species. A polyphasic study was undertaken to determine the taxonomic position of these six isolates. The strains were characterized by 16S rRNA and AtpA sequence analysis and by conventional phenotypic testing. The whole genome sequences were determined for all isolates and the average nucleotide identity (ANI) was determined. The isolates formed a separate phylogenetic clade, divergent from all other *Campylobacter* taxa and most closely related to *C. mucosalis*. Although all isolates showed 100% 16S rRNA sequence homology, AtpA and ANI analyses indicated divergence between the otariid isolates from California and the phocid isolates from Scotland, which warrants subspecies status for each clade. The two subspecies can also be distinguished phenotypically based on catalase activity. This study shows clearly that the isolates obtained from pinnipeds represent a novel species within the genus *Campylobacter*, for which the name *Campylobacter pinnipediorum* sp. nov. is proposed. Within this novel species, the Californian isolates represent a separate subspecies, for which the name *C. pinnipediorum* subsp. *pinnipediorum* subsp. nov. is proposed. The type strain for both this novel species and subspecies is RM17260^T (= LMG 29472^T = CCUG 69570^T). The Scottish isolates represent another subspecies, for which the name *C. pinnipediorum* subsp. *caledonicus* subsp. nov. is proposed. The type strain of this subspecies is M302/10/6^T (= LMG 29473^T = CCUG 68650^T).

Of all currently recognized *Campylobacter* species, at least five are predominantly associated with animals found in marine environments. *Campylobacter lari*, *C. peloridis*, *C. subantarcticus* and *C. volucris* (Debruyne *et al.*, 2009, Debruyne *et al.*, 2010a, Debruyne *et al.*, 2010b) are all isolated from shellfish and/or marine birds, whereas *C. insulaenigrae* has been isolated from marine mammals (Foster *et al.*, 2004). These predominantly thermotolerant species are all closely related and belong to the same clade, which includes the human pathogen *C. jejuni*. In contrast, *Campylobacter* species from the clade to which *C. concisus* and *C. mucosalis* belong are not or are rarely associated with marine animals; many are isolated from the oral cavity of terrestrial vertebrates, including humans (Debruyne *et al.*, 2008). We describe a novel, urease positive *Campylobacter* species, related to *C. mucosalis* and *C. concisus*, which has been isolated from pinnipeds. Furthermore, this species is proposed to contain two subspecies.

Pinnipeds comprise the families Odobenidae (walruses), Otariidae (eared seals) and Phocidae (earless seals). Six *Campylobacter*-like isolates not belonging to any of the established *Campylobacter* taxa were obtained from pinnipeds during independent diagnostic screenings in California (United States) and Scotland (United Kingdom). Three isolates were obtained from internal organs and an abscess of California sea lion (*Zalophus californianus*) juveniles from a seal rehabilitation centre in San Diego. Three isolates were obtained from abscesses of stranded deceased common seal (*Phoca vitulina*) and grey seal (*Halichoerus grypus*) juveniles from the coastal regions of Scotland. Noteworthy, from two of these abscesses no organisms other than the *Campylobacter*-like organisms were isolated.

Initial characterization using AFLP (Duim *et al.*, 1999) and 16S rRNA sequencing (Maiwald, 2004) indicated that these isolates were different from all other *Campylobacter* species, but

most closely related to *C. mucosalis*. A recent study analysing the microbiota of sea mammals (Bik *et al.*, 2016) identified *Campylobacter* 16S rRNA sequences in both oral and gastric samples from California sea lions, but not from common bottlenose dolphins (*Tursiops truncatus*). These 16S rRNA sequences shared 99-100% sequence homology with the 16S rRNA sequences of the isolates described in this study.

A polyphasic study was undertaken to determine the taxonomic position of these six isolates. Whole genome sequencing was performed on all isolates and the average nucleotide identities (ANI) were determined. Comparisons based on 16S rRNA gene and AtpA protein sequences were made to determine the taxonomic position of the isolates. Phenotypic characteristics were determined by conventional biochemical testing for all six isolates.

Apart from the six isolates used for extended taxonomic analysis, five additional *Campylobacter* isolates with identical 16S rRNA sequences were obtained from the oral and rectal cavities of California sea lions and from an abscess of a Steller sea lion (*Eumetopias jubatus*). In support of the extended taxonomic analysis these isolates were used for ANI and evaluated on the discriminating phenotypic tests. Characteristics of all strains are summarized in Table 1.

Complete genome sequences for strains RM17260, RM17261, RM17262 and M302/10/6 were obtained as described (Miller *et al.*, manuscript in preparation). Briefly, initial sequencing was performed on a Roche 454 GS-FLX+ Genome Sequencer (Roche Life Science, Indianapolis, IN). 454 sequencing reads were assembled into single scaffolds using the Roche Newbler assembler (ver. 2.6) and base calls were validated using Illumina MiSeq (Illumina Inc., San Diego, CA) reads. Additional sequencing was performed for the above four strains and de novo for strains M341/11/05 and M203/00/3 using a PacBio RS sequencer (Pacific Biosciences, Menlo Park, CA) to generate complete, closed genomes. Draft genomes for strains RM18812, RM18813, RM18906, 1105248A and 03036546 were obtained using the Illumina MiSeq. Sequencing reads were assembled using Newbler or SPAdes (ver. 3.1.1). The genome sequences have been deposited in GenBank; accession numbers are listed in Table 1.

The taxonomic position of all strains was determined by 16S rRNA gene comparison. The 16S rRNA gene sequences (≥ 1339 bp) were extracted from the whole genome sequences of the strains or obtained from EzTaxon (Kim *et al.*, 2012) for the other *Campylobacter* species. Sequence alignment and dendrogram construction were performed using CLUSTALX (ver. 2.1) and MEGA version 6.05 (Tamura *et al.*, 2013). A neighbour-joining dendrogram containing all *Campylobacter* taxa was constructed (Fig. 1). Bootstrap values were determined using 500 repetitions. The 16S rRNA gene sequence from *Arcobacter butzleri* strain RM4018 was used to root the tree. The 16S rRNA gene sequence similarity between the pinniped-associated strains was 100%, while the sequence similarity between these strains and the most closely related species *C. concisus* and *C. mucosalis* was 96-97%.

For improved taxonomic resolution (Miller *et al.*, 2014a), full AtpA protein sequences were

extracted from the whole genome sequences or obtained from GenBank. Alignment and dendrogram construction were performed as described above; the AtpA sequence from *Arcobacter butzleri* strain RM4018 was used to root the tree. Consistent with the 16S rRNA comparison, the pinniped-associated strains formed a clade distinct from other *Campylobacter* taxa (Fig. 2). Furthermore, a clear distinction could be observed between the strains isolated in California and Scotland.

As an alternative for DNA-DNA hybridization (DDH), the average nucleotide identity (ANI) has been suggested (Konstantinidis & Tiedje, 2005, Konstantinidis *et al.*, 2006). A DDH species delineation of 70% corresponds to about 95% ANI (Goris *et al.*, 2007). Using the JSpecies v. 1.2.1 (Richter & Rosselló-Móra, 2009), pair-wise ANI values based on whole genome sequences were calculated for all the unidentified *Campylobacter* strains and the most closely related species: *C. concisus* (strain 13826; accession no. CP000792), *C. curvus* (strain 525.92; accession no. CP000767), *C. mucosalis* (strain DSM 21682^T; accession no. JHQQ01), *C. rectus* (strain ATCC 33238^T; accession no. ACFU00000000) and *C. showae* (strain ATCC 51146^T; accession no. ACVQ00000000). While ANI for digital DDH assessments of *Campylobacter* and related species has not been tested, the ANI between the pinniped-associated strains and most closely related species (*C. mucosalis*) was maximally 71%, which is well below the 95% species cut-off suggested by Goris and colleagues (2007) and similar to ANI values observed between the pinniped-associated strains and the other related species (Table 2). Strains originating from either California or Scotland were highly homologous amongst each other (ANI $\geq$ 98%). However, 94-95% ANI was observed between the Californian and Scottish strains, indicating divergence between both groups of strains on a genomic level. The taxonomic position of the novel *Campylobacter* strains is

further supported by a core genome phylogeny which includes these strains and related *Campylobacter* taxa (Miller *et al.*, manuscript in preparation).

The genetic analyses presented here indicate that the pinniped-associated strains form a distinct clade which is clearly separated from the closest known relatives. Based on the 100% 16S rRNA homology, all strains examined clearly belong to the same species. However, the divergence observed between the Californian and Scottish strains, based on the ANI, AtpA and core genome phylogeny, warrants subspecies status for each group. The 94-95% ANI observed between the Californian and Scottish strains is consistent with the subspecies divergence observed in other *Campylobacter* species, such as *C. fetus* (8% divergence between *C. fetus* subsp. *fetus* and *C. fetus* subsp. *testudinum*) and *C. hyointestinalis* (6% divergence between *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp. *lawsonii*) (Miller *et al.*, 2016).

The G+C content was determined based on the whole genome sequences using Artemis v.13.2 (Wellcome Trust Sanger Institute, UK; Rutherford *et al.*, 2000). All strains had a G+C content varying between 30.4% and 31.0%, which is within the range observed in *Campylobacter* (Table 3).

Additional phenotypic testing was performed as described previously (On & Holmes, 1991a, On & Holmes, 1991b, On & Holmes, 1992, Ursing *et al.*, 1994). Oxidase activity, catalase activity, nitrate reduction, indoxyl acetate hydrolysis, urea hydrolysis, hippurate hydrolysis and H_2S production on TSI agar were determined. In addition to this, growth with 1% glycine, α-haemolysis on sheep blood agar, H_2 requirement and resistance to nalidixic acid (30 μg) and cephalothin (30 μg) were evaluated. Strains were grown at various temperatures,

atmospheres and on different agar media. The strains displayed phenotypic characters distinct from all other *Campylobacter* taxa. All strains displayed urease activity, which may be related to a gastric niche. Indeed, 99-100% 16S rRNA sequence homology was observed between the strains and uncultured bacteria from the gastric microbiota of California sea lions (Bik *et al.*, 2016). Differentiating characteristics for the strains tested and other *Campylobacter* taxa are summarized in Table 3. Based on urease activity, H_2S production on TSI agar, nitrate reduction, growth at 25°C in a microaerobic atmosphere and α-haemolysis, the strains can be distinguished from all other described *Campylobacter* taxa. Results of the discriminatory phenotypic tests were identical for the five additional *Campylobacter* strains. Catalase activity was observed in all strains originating from otariid seals in California, but not in strains originating from phocid seals in Scotland, supporting the existence of two subspecies. Urease-positive *Campylobacter lari* (UPTC) are not, as yet, a defined taxon within *Campylobacter* (Bolton et al., 1985, Megraud et al., 1988, Endtz et al., 1997, Debruyne et al., 2009). Nevertheless, since such strains are also urease positive they could potentially share the same phenotypic profile as the pinniped-associated strains. Therefore, their phenotypic characteristics were also analysed, based on *C. lari* strains NCTC 11845, CCUG 22395, RM16701 and RM16712 (Miller *et al.*, 2014b). UPTC strains could not grow at 25°C microaerobically and did not produce H_2S on TSI agar; thus, the pinniped-associated strains could also be readily distinguished from the urease positive *C. lari*.

In conclusion, the results from this polyphasic taxonomic study clearly demonstrate that the isolates recovered from pinnipeds comprise a novel species distinct from all other currently known *Campylobacter* species, based on 16S rRNA, AtpA, whole genome sequence comparison and biochemical properties. The name *Campylobacter pinnipediorum* sp. nov. is proposed for these strains. Within this novel species, strains originating from otariid seals in California form a separate subspecies, for which the name *C. pinnipediorum* subsp. *pinnipediorum* subsp. nov. is proposed. Strains originating from phocid seals in Scotland form another subspecies, for which the name *C. pinnipediorum* subsp. *caledonicus* subsp. nov. is proposed.

Description of *Campylobacter pinnipediorum* sp. nov.

Campylobacter pinnipediorum (pin.ni.pe.di.o'rum. N.L. gen. pl. n. pinnipediorum, pertaining to Pinnipedia).

Gram-negative slightly curved to spiral-shaped rods. After incubation on Columbia agar with 5% sheep blood in a microaerobic atmosphere at 37°C for 72 h colonies appear transparent to beige, glossy, slightly raised and circular with smooth margins. A clear dimorphic growth was observed: the majority of colonies are small (< 0.5 mm), flat and appear transparent to beige; however, a minority formed larger (0.5-1 mm), slightly raised, whitish and translucent colonies, which show α-haemolysis. After a week of growth at 37°C in a microaerobic atmosphere colonies are 2-3 mm and appear circular with smooth margins, whitish, translucent, with greenish periphery due to α-haemolysis; however, in an anaerobic atmosphere colonies appear circular with smooth margins, transparent with a whitish centre and radiating from the centre, while α-haemolysis is absent. Shows no growth at aerobic conditions. No H_2 is required for growth at microaerobic conditions. In a microaerobic atmosphere, growth is observed after 48 h at 25°C and 37°C, but not at room temperature (18-22°C) or 42°C. All strains produced H_2S on TSI agar and were positive for urea hydrolysis, oxidase activity and nitrate reduction, but were negative for hydrolysis of hippurate and indoxyl acetate. Catalase activity is variable. In a microaerobic atmosphere at 37°C normal growth on Skirrow agar, but no growth on charcoal cefoperazone deoxycholate (CCD) agar and no or limited growth on IST agar, Mueller-Hinton agar nor in the presence of 1% glycine. All strains were susceptible to cephalothin and nalidixic acid. Pathogenicity is unknown, although an association with infection is observed, as most currently known isolates have been recovered from abscesses and internal organs of pinnipeds. The species

type strain is RM17260^{T} (= LMG 29472^{T} = CCUG 69570^{T}), which was isolated from an abscess of a California sea lion (*Zalophus californianus*) in 2013.

Description of *Campylobacter pinnipediorum* sp. nov. subsp. *pinnipediorum* subsp. nov.

Campylobacter pinnipediorum subsp. *pinnipediorum* (pin.ni.pe.di.o'rum. N.L. gen. pl. n. pinnipediorum, pertaining to Pinnipedia).

The strains adhere to the species description as given above. This subspecies can be distinguished from *Campylobacter pinnipediorum* sp. nov. subsp. *caledonicus* subsp. nov. by divergent AtpA sequence and production of catalase. Pathogenicity is unknown, although an association with infection is observed, as most currently known isolates have been recovered from abscesses and internal organs of California sea lions (*Zalophus californianus*) and a Steller sea lion (*Eumetopias jubatus*). The subspecies type strain is RM17260^{T} (= LMG 29472^{T} = CCUG 69570^{T}), which was isolated from an abscess of a California sea lion (*Zalophus californianus*) in 2013.

Description of *Campylobacter pinnipediorum* sp. nov. subsp. *caledonicus* subsp. nov.

Campylobacter pinnipediorum subsp. *caledonicus* (ca.le.do'ni.cus. L. masc. adj. caledonicus, from Caledonia (Scotland), the geographic area where the organism has been isolated).

The strains adhere to the species description as given above. This subspecies can be distinguished from *Campylobacter pinnipediorum* sp. nov. subsp. *pinnipediorum* subsp. nov. by divergent AtpA sequence and the lack of catalase activity. Pathogenicity is unknown, although an association with infection is observed, as all currently known isolates have been recovered from abscesses of common seals (*Phoca vitulina*) or grey seal (*Halichoerus*

247 *grypus*). The subspecies type strain is M302/10/6^{T} (= LMG 29473^{T} = CCUG 68650^{T}), which

248 was isolated from a lung abscess of a grey seal (*Halichoerus grypus*) in 2010.

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Figure legends & tables

Figure 1. Neighbor-joining phylogenetic dendrogram based on 16S rRNA gene sequences. Bootstrap values (≥ 75%) based on 500 replications are indicated at the nodes.

Figure 2. Neighbor-joining phylogenetic dendrogram based on AtpA protein sequences. Bootstrap values (≥ 75%) based on 500 replications are indicated at the nodes. *C. pinnipediorum* genome sequence accession numbers are as listed in Fig. 1.

352 Table 1. Features of the *C. pinnipediorum* sp. nov. strains used in this study.

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Subspecies	Strain	Alternative designation	Isolation date	Location	Host species	Source	Sex	Age	Accession no.
C. p. subsp. *pinnipediorum*	RM17260^T	LMG 29472^T CCUG 69570^T SW130133	7-2-2013	San Diego, California, US	California sea lion (*Zalophus californianus*)	Abscess	n/a	Juvenile	CP012546
C. p. subsp. *pinnipediorum*	RM17261	SW130167	n/a	San Diego, California, US	California sea lion (*Zalophus californianus*)	Lung	n/a	Juvenile	CP012547
C. p. subsp. *pinnipediorum*	RM17262	SW130202	n/a	San Diego, California, US	California sea lion (*Zalophus californianus*)	Abscess fluid	n/a	Juvenile	CP012548
C. p. subsp. *pinnipediorum*	RM18812	SWCZC1617B	1-25-2016	San Diego, California, US	California sea lion (*Zalophus californianus*)	Oral	F	Juvenile	MDCT00000000
C. p. subsp. *pinnipediorum*	RM18813	SWCZC1626B	1-29-2016	San Diego, California, US	California sea lion (*Zalophus californianus*)	Rectal	F	Juvenile	MDCU00000000
C. p. subsp. *pinnipediorum*	RM18906	SWCZC1639B	2-27-2016	San Diego, California, US	California sea lion (*Zalophus californianus*)	Oral	M	Juvenile	MDCV00000000
C. p. subsp. *pinnipediorum*	1105248A	16S02911-1	5-2011	Laguna Beach, California, US	California sea lion (*Zalophus californianus*)	Abscess	n/a	n/a	MCRK00000000
C. p. subsp. *pinnipediorum*	0306546	16S02912-1	3-9-2003	Resurrection Bay, Alaska, US	Steller sea lion (*Eumetopias jubatus*)	Abscess	F	Juvenile	MCRL00000000
C. p. subsp. *caledonicus*	M203/00/3	n/a	3-11-2000	Inverness, Scotland, UK	Common seal (*Phoca vitulina*)	Shoulder abscess	M	Juvenile	MBGA00000000
C. p. subsp. *caledonicus*	M302/10/6	LMG 29473 CCUG 68650	11-15-2010	Inverness, Scotland, UK	Grey seal (*Halichoerus grypus*)	Lung abscess	F	Juvenile	CP017018
C. p. subsp. *caledonicus*	M341/11/05	n/a	11-29-2011	Inverness, Scotland, UK	Common seal (*Phoca vitulina*)	Submaxillary abscess	F	Juvenile	CP017258

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Table 2. Average nucleotide identity (ANI) values (%) based on BLAST for *C. pinnipediorum* sp. nov. and the most closely-related *Campylobacter* species.

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	100	98	98	98	98	97	98	98	94	94	94	70	68	71	67	67
2	98	100	98	98	98	97	98	98	94	94	94	70	68	71	66	67
3	98	98	100	98	98	97	98	98	95	94	94	70	68	71	66	67
4	98	98	98	100	98	97	98	98	94	94	94	69	68	71	66	67
5	98	98	98	98	100	97	98	98	94	94	94	69	68	71	66	67
6	97	97	97	97	97	100	97	97	95	95	95	69	68	71	66	67
7	98	98	98	98	98	97	100	98	94	94	94	69	68	71	66	66
8	98	98	98	98	98	97	98	100	94	94	94	69	68	71	66	67
9	94	94	94	94	94	95	94	94	100	99	99	69	68	71	66	67
10	94	94	94	94	94	95	94	94	99	100	99	69	68	71	66	67
11	94	94	94	94	94	95	94	94	99	99	100	69	68	71	66	67
12	69	69	69	70	69	70	69	69	69	69	69	100	74	71	71	71
13	68	68	68	68	68	68	68	68	68	68	68	74	100	70	72	72
14	71	71	71	71	71	71	71	71	71	71	71	71	70	100	68	69
15	66	66	66	66	66	66	66	66	66	66	66	71	72	69	100	89
16	67	67	67	67	67	67	67	67	67	67	67	71	72	69	89	100

Strains: 1, *C. pinnipediorum* sp. nov. RM17260^{T} ; 2, *C. pinnipediorum* sp. nov. RM17261; 3, *C. pinnipediorum* sp. nov. RM17262; 4, *C. pinnipediorum* sp. nov. RM18812; 5, *C. pinnipediorum* sp. nov. RM18813; 6, *C. pinnipediorum* sp. nov. RM18906; 7, *C. pinnipediorum* sp. nov. 1105248A; 8, *C. pinnipediorum* sp. nov. 0306546; 9, *C. pinnipediorum* sp. nov. M302/10/6; 10, *C. pinnipediorum* sp. nov. M341/11/05; 11, *C. pinnipediorum* sp. nov. M203/00/3; 12, *C. concisus* 13826; 13, *C. curvus* 525.92; 14, *C. mucosalis* ATCC 49352^{T}; 15, *C. rectus* ATCC 33238^{T}; 16, *C. showae* ATCC 51146^{T}. Strains 1-8, *C. pinnipediorum* subsp. *pinnipediorum* subsp. nov.; strains 9-11, *C. pinnipediorum* subsp. *caledonicus* subsp. nov.

367 Table 3. Characteristics differentiating *C. pinnipediorum* sp. nov. from other taxa of the *Campylobacter* genus.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
Oxidase	+	+	+	+	+	V	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	V	+	+	+	+	+
Catalase	+	-	+	V	+	-	+	+	-	+	+	(+)	(-)	-	-	+	+	+	+	V	+	+	+	+	-	+	(-)	+	V*	+	-	V	+
Urease	+	+	-	V	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	-	V*	ND	-	+	ND
Nitrate reduction	+	+	+	V	+	(-)	(+)	+	+	+	+	(+)	(+)	+	-	+	+	+	+	-	+	+	+	+	(-)	ND	+	+	(+)	+	+	+	+
Hippurate hydrolysis	-	-	+	-	-	-	-	-	(-)	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Indoxyl acetate hydrolysis	-	-	+	-	+	-	V	+	V	-	-	-	(+)	+	-	-	-	-	-	+	+	-	ND	-	-	ND	+	V	-	-	+	V	-
γ-Glutamyl transferase	+	+	-	+	-	-	ND	-	ND	-	ND	-	ND	-	ND	-	-	+	ND	-	-	ND	ND	-	ND	ND	ND	ND	-	ND	-	ND	ND
H_2S production (TSI)	+	+	-	V	-	-	+	-	(-)	-	-	-	-	-	-	+	+	+	-	-	-	-	ND	-	+	ND	-	V	+	-	-	-	-
α-Haemolysis	+	+	-	-	(-)	(-)	-	+	(-)	-	-	V	-	+	-	V	V	+	ND	+	+	+	ND	+	-	ND	+	+	+	+	+	V	ND
Growth at/in/on:																																	
18-22°C (microaerobic)	-	-	ND	ND	-	-	ND	ND	-	(+)	+	(-)	-	-	ND	(-)	-	+	ND	-	-	ND	ND	-	-	ND	-	-	-	-	-	ND	-
25°C (microaerobic)	+	+	-	-	-	-	ND	-	-	+	+	+	-	-	-	(-)	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37°C (microaerobic)	+	+	+	+	+	+	+	+	V	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	V	+	+	+	+	+
42°C (microaerobic)	-	-	+	+	+	(+)	ND	(+)	V	(+)	+	-	V	+	(-)	+	+	-	-	-	+	+	+	+	+	+	(-)	V	+	+	+	V	+
37°C (anaerobic)	+	+	-	+	-	+	+	-	+	(-)	+	V	+	-	+	-	+	+	-	-	-	+	ND	-	+	ND	+	+	+	+	-	+	+
37°C (aerobic)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CCDA	-	-	-	+	+	(-)		(+)	(+)	+	+	+	V	+	ND	+	+	+	ND	+	+	ND	+	+	+	+	-	+	(+)	ND	+	ND	ND
Glycine (1%)	V	-	-	V	(+)	(-)	+	-	+	+	+	(-)	+	V	+	+	V	+	+	(-)	+	-	(+)	+	(-)	+	+	V	+	(+)	+	+	-
Resistance to:																																	
Nalidixic acid (30 μg)	-	-	-	V	-	(+)	+	V	+	+	+	V	V	-	V	+	+	+	+	-	-	+	-	(+)	(+)	(+)	(+)	-	(+)	+	-	-	+
Cephalothin (30 μg)	-	-	+	-	+	-	-	(+)	-	-	ND	-	-	-	-	(-)	-	-	+	-	+	+	+	+	-	(-)	-	-	-	-	(-)	-	+
H_2 requirement	-	-	V	-	-	+	-	-	+	-	ND	-	+	-	+	V	V	-	ND	-	-	-	ND	-	+	ND	+	+	-	ND	-	+	ND
DNA G+C content (mol%)	30	31	35	ND	31	37-41	32	32	45-46	33-35	33	33-34	44-46	34	32-33	35-36	31-33	36	ND	31	30-31	36	30	29-30	36-38	29	45-46	44-46	29-33	30	32-36	28-30	29

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Taxa: 1, *C. pinnipediorum* subsp. *pinnipediorum* subsp. nov. (*n* = 3); 2, *C. pinnipediorum* subsp. *caledonicus* subsp. nov. (*n* = 3); 3, *C. avium*; 4, *C. canadensis*; 5, *C. coli*; 6, *C. concisus*; 7, *C. corcagiensis*; 8, *C. cuniculorum*; 9, *C. curvus*; 10, *C. fetus* subsp. *fetus*; 11, *C. fetus* subsp. *testudinum;* 12, *C. fetus* subsp. *venerealis*; 13, *C. gracilis*; 14, *C. helveticus*; 15, *C. hominis*; 16, *C. hyointestinalis* subsp. *hyointestinalis*; 17, *C. hyointestinalis* subsp. *lawsonii*; 18, *C. iguaniorum*; 19, *C. insulaenigrae*; 20, *C. jejuni* subsp. *doylei*; 21, *C. jejuni* subsp. *jejuni*; 22, *C. lanienae*; 23, *C. lari* subsp. *concheus*; 24, *C. lari* subsp. *lari*; 25, *C. mucosalis*; 26, *C. peloridis*; 27, *C. rectus*; 28, *C. showae*; 29, *C. sputorum*; 30, *C. subantarcticus*; 31, *C. upsaliensis*; 32, *C. ureolyticus*; 33, *C. volucris*. Characteristics of reference taxa were adapted from previous species descriptions (Vandamme *et al.*, 2010, Koziel *et al.*, 2014, Gilbert *et al.*, 2015). +, 90-100%; (+), 75-89%; V, 26-74%; (-), 11-25%; -, 0-10%; ND, not determined; *, test results differ between *C. sputorum* biovars *sputorum* (catalase and urease negative), *paraureolyticus* (catalase negative, urease positive) and *fecalis* (catalase positive, urease negative).